GENETIC DETECTION OF AMINOGLYCOSIDE-RESISTANCE GENE AMONG KLEBSIELLA PNEUMONIAE ISOLATED FROM ENVIRONMENTAL OF AL-NAJAF HOSPITALS, IRAQ

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Abstract- Klebsiella pneumoniae is one of the most medical microorganisms that able to cause serious infections. This pathogen a growing concern public health and may be leads to the mortality. The aim of the current research was to investigate and determine aminoglycoside genes resistant as well as antimicrobial agents among isolates of K. pneumoniae isolated from hospital environmental. the current study were revealed among 400 swabs isolated from various origin of hospital environmental only 103(25.75%) were gram negative bacterial isolates, while 28(7%) diagnosis as K. Pneumoniae isolates. Ruslts of antibiogram profile showed concern level of resistance 13(46.42%) of K. Pneumoniae isolates were multi-drug resistance (MDR) compared with 8(28.57%) were extensive-drug resistance (XDR). at same matter, all isolates were sensitive to impenem and meropenem antibiotics. Moreover, The data of Multiple antibiotic resistance index indicate to a huge antibacterial agents used in the hospital environment. Molecular data were revealed a high strA-B gene among K. Pneumoniae isolates reached to 20/28(%).compared with 2/28(%) and 1/28(%) for aacC-1 and aac(6′)/aph(2′) genes respectively. At same time, aacC-2, aph(3′)-IIIa and ant(4′)-Ia were no observed in this study.

I. INTRODUCTION

Klebsiella pneumoniae is one of the most medical microorganisms that able to cause serious infections. This pathogen a growing concern public health and may be leads to the mortality. Klebsiella genus is in charge of vast assortment of infections, such as urinary tract infection, neonatal sepsis, pneumonia, burn and wound diseases. K. pneumoniae promptly clings to medical devices, in this manner expanding danger of contamination for patients experiencing strategies with reused or embedded therapeutic hardware and immunocompromised people are particularly defenseless(Li et al., 2014; Vading et al., 2018).

Beta-lactam drug are a main category of antimicrobial agents recommended in human medication. Moreover, a wide used to treated enterobacteriaceae including Klebsiella, On account of the expanding beta-lactam resistance of enteric pathogen, aminoglycoside anti-microbial agents are getting to be essential treatment alternatives for multi-drug resistance bacteria (Shlash and tuwaij, 2018). Therefore,The current study aimed at detecting the spread of K. pneumoniae isolates in hospital environments as well as determining the type of genes responsible for the resistance of these bacteria to the aminoglycoside drugs.

II. MATERIALS AND METHODS

All experiments of current study were done in Advance laboratories of Department of Biology, Faculty of Science, University of Kufa.

Sample Collection and bacterial identification

This study included the collection of 400 swabs from hospital environments (Al-Hakeem Hospital and Medical Al-Sader City) as well as the center of the burns unit in Al-Najaf City, as follows (Glove and mask, Apparatus and tools, Bathroom, Wall and floor and bedding of patients) with 80 swabs per source during period November 2018 to January 2019. Swabbing of the samples were achieved through use of sterile cotton swabs wet by sterile saline. Range of the surfaces of tested area swabbed from 5 to 10 cm^2 then immediately cultured on sterile MacConkey agar medium (oxoid,Uk) and it incubated aerobically at 37 Cº(MacFaddin,2000).

All growing bacteria were again streaking on sterile MacConkey agar plate (oxoid,Uk) to obtain pure single colonies. Some biochemical tests, motility and oxidase test were depending to diagnosis klebsiella genus and Vitek-2 system to confirm diagnosis of K. pneumoniae isolates.

Antimicrobial susceptibility test

It achieved depending on criteria of clinical and Laboratory Standards Institute (2017), using method of Kirby-Bauer (Baure and Kirby,1966). Each isolates were suspending according to 0.5 McFarland standard tube and immediate lawn on sterile muller hinton agar plate. This test included 15 commercial antibiotic disks (Bioanalyse, Tyrkey) return to 6 classes of antimicrobial agent (describe below in tabe 4).

Estimation of Index of Multiple Antibiotic Resistance (MAR)

The index of multiple antibiotic resistance (MAR) was calculable for every isolates through the equation MAR = a / b, wherever the letter (a) meaning the amount of antibiotics that the microorgaism shown resistance , whereas the letter (b) meaning the whole range of antibiotics to that the tested isolates were estimated for antimicrobial susceptibility. Each

pathogen that has MAR index more or equal to 0.2 produce from a great hazard source of contamination wherever many antibacterial agent are used (Sandhu et al., 2016).

Molecular assay

Total DNA extraction

Whole genomic of all 28 K. pneumoniae isolates were extracted using Genomic DNA Mini Plasmid Kit (Geneaid Biotech, South Korea) be based on steps of instructions of the manufacture. DNA were apply to PCR apparatus for detection aminoglycoside genes using specific primer(table 1) according conditions listed in table (2), the product of PCR was separated using electrophoresis (Bioneer, Korea) then band was seen used gel documented system (Cleaver, UK).

Table (1): Sequence of primers used in the current study

Gene	Name	Sequence (5´ to 3´)	Product size(bp)	Reference
aacC-1	aacC-1-F	ATGGGCATCATTCGCACATGTAGG	873	Hujer et al.,(2006)
	aacC-1-R	TTAGGTGGCGGTACTTGGGTC		
aacC-2	aacC-2-F	ATGCATACGCGGAAGGCAATAAC	861	
	aacC-2-R	CTAACCGGAAGGCTCGCAAG		
strA-strB	strA-F	ATGGTGGACCCTAAAACTCT	893	Duran et al.,(2012)
	strA-R	CGTCTAGGATCGAGACAAAG		
aac(6′)/aph(2′)	aac(6′)/aph(2′)-F	GAAGTACGCAGAAGAGA	491	
	aac(6′)/aph(2′)-R	5′-ACATGGCAAGCTCTAGGA		
aph(3′)-IIIa	aph(3′)-IIIa-F	5′-AAATACCGCTGCGTA-3′	242	
	aph(3′)-IIIa-R	5′-CATACTCTTCCGAGCAA-3′		
ant(4′)-Ia	ant(4′)-Ia-F	5′-AATCGGTAGAAGCCCAA-3′	135	
	ant(4′)-Ia-R	5′-GCACCTGCCATTGCTA-3′		

Table (2): Conditions of PCR thermo cycling

monoplex gene	Temperature (c) / Time					Cycle number
	Initial denaturation	Cycling condition			Final extension	
		Denaturation	Annealing	Extension		
aacC-1	94 C° /5min	94 C°/1 min	55 C°/1 min	72 C°/ 2min	72 C°/ 5min	30
aacC-2	94 C°/5min	94 C°/1 min	55 C°/1 min	72 C°/ 2min	72 C°/ 5min	30
strA-strB	94 C°/10min	94 C°/1 min	54 C°/1 min	72 C°/ 1min	72 C°/10 min	30
aac(6′)/aph(2′)	94 C°/5min	94 C°/1 min	55 C°/1 min.	72 C°/ 1 min	72 C°/ 7min	30
aph(3′)-IIIa	94 C°/5min	94 C°/1 min	55 C°/1 min.	72 C°/ 1 min	72 C°/ 7min	30
ant(4′)-Ia	94 C°/5min	94 C°/1 min	55 C°/1 min.	72 C°/ 1 min	72 C°/ 7min	30

RESULT AND DISCUSSION

The current study was revealed among 400 swabs taken from different source of hospital environmental 103(25.75%) were gram negative bacterial isolates. According to phenotypic and biochemical characteristic as well as Vitek-2 system the data demonstrated out of 103 gram negative bacterial isolates were 28(7%) identified as K. Pneumoniae isolates (table 3). Many local and international studies indicate that bacteria are present in hospital environments (Al-Sehlawi, 2012). Locally, a study was conducted by Al-Shamamri and Al-Khattat, (2015), who showed that out of 245 specimens(159 clinical and 50 hospital environment) taken from three hospitals in Najaf city, were isolated 89 K. pneumoniae.

Table (3): Distribution of K. Pneumoniae isolates among hospital environmental sources

Sample sources	Number of samples	Number of gram negative growth	Number of K. Pneumoniae
Glove and mask	80	0(0%)	0(0%)
Apparatus and tools	80	8(10%)	2(2.5%)
Bathroom	80	43(53.75)	10(12.5%)
Wall and floor	80	21(26.25%)	5 (6.25%)
bedding	80	31(38.75%)	11(13.75%)
Total	400	103(25.75%)	28 (7%)

Anti-bigram profile

The data in table (4) indicated that isolates K. Pneumoniae has been various degree of resistance among tested antimicrobial agents started with high resistance for Ceftriaxone27(96.42%), Amoxicillin and Ceftazidame were 24(85.71%), Also this

pathogen gave moderate resistance such as Cefepeme and Streptomycin were 18(64.28%), while aminoglycoside agents including Tetilmicin, Gentamicin and amikacin were appeared good effect against isolates K. Pneumoniae except streptomycin was less resistnace among K. Pneumoniae isolates reached to 18(64.28%). The results also demonstrated that Carbapenem antibiotics (Impenem and meropenem) were best choice to threating this microorganism where all isolates were sensitive to this drug. Locally, there are several studies revealed that K. Pneumoniae isolate has vast spectrum of resistance to commercial antimicrobial agent that due to mise use of drug as well as self-treatment without formal a recipe by physician (Al-Sehlawi, 2012 ; Shlash and tuwaij, 2018).

Table (4): antibiogram profile of K. Pneumoniae isolates

Antimicrobial agent	Resistance(%)	Intermediate (%)	Sensitive (%)
carbenicillin (PY, 25mg)	19(67.85%)	5(17.85%)	4(14.28%)
Amoxicillin (AX, 10mg)	24(85.71%)	1(3.57%)	3(10.71%)
Cefoxitn (FOX, 30 mg)	21(75%)	2(7.14%)	5(17.85%)
Ceftriaxone (CRO, 30 mg)	27(96.42%)	0(0%)	1(3.57%)
Ceftazidame (CAZ, 30 mg)	24(85.71%)	2(7.14%)	2(7.14%)
Cefepeme (FEP, 30 mg)	18(64.28%)	1(3.57%)	9(32.14%)
Aztreonam (ATM, 30 mg)	21(75%)	4(14.28%)	310.71(%)
Levofloxacin (LEV, 5 mg)	7(25%)	3(10.71%)	18(64.28%)
Ciproflaxacin (CIP,10 mg)	8(28.57%)	0(0%)	20(71.42%)
Tetilmicin (NET, 10 mg)	8(28.57%)	3(10.71%)	17(60.71%)
Streptomycin (S, 10 mg)	18(64.28%)	2(7.14%)	8(28.57%)
Gentamicin (CN, 10 mg)	11(39.28%)	3(10.71%)	14(50%)
Amikacin (AK, 10 mg)	13(46.42%)	1(3.57%)	14(50%)
Impenem (IMP, 10 mg)	0(0%)	0(0%)	0(0%)
Meropenem (MEM, 10 mg)	0(0%)	0(0%)	0(0%)

The data in current study were indicated that there are a high percentage 13 (46.42%) of these bacteria resistant to at least three categories of antibiotics so adopted as MDR compared with 8(28.57%) were XDR (Resistant to all antibiotics except one or two class). While 7(25%) was Resistance to one or two classes(table5). Elevation of MDR and XDR among environmental K. pneumoniae is an indicator of concern. So should be apply antimicrobial surveillance .

Table (5): Determination of MDR, XDR and PDR among isolates of K. Pneumoniae isolated from hospital environment

Type of resistance	Number of isolates
MDR	13(46.42%)
XDR	8(28.57%)
PDR	0(0%)
Resistance to one or two classes	7(25%)
Total	28(100%)

Index of Multiple Antibiotic Resistance (MAR)

The analysis of MAR indexes among K. peumoneae isolates were ranged from 0.1to 0.8. The data revealed a huge antibacterial agents use in the environment of hospital (table 6). However, the results revealed that Frequency of MAR Index were at 0.6 and 0.4 reached to 6 isolates for both.

Table (6): Multiple antibiotic resistance index for isolates of K. peumoneae

MAR Index	Frequency of MAR Index
0.1	1
0.2	3
0.3	5
0.4	6
0.5	0
0.6	6
0.7	3
0.8	4
0.9	0
1.0	0

The data in figures(1,2 and 3) about PCR amplification of aminoglycoside-resistant genes were revealed that high rate of strA-B gene among K. Pneumoniae isolates reached to 20/28(%).Moreover, out of 28 K. Pneumoniae isolates were 2/28(%) and 1/28(%) K. Pneumoniae isolates for aacC-1 and aac(6′)/aph(2′) genes respectively. At same time, aacC-2, aph(3′)-IIIa and ant(4′)-Ia were no detected in this study. This data was low from a local study conducted in the city of Al-Najaf by Al-Janabi and Al Hasnawi (2017) pointed out that the proportion of aacC-1 gene was 12(27.90%) compared with 2(9.30%) among 43 isolates of K. pnuemoniae.

There are limited or low study on aph(3′)-IIIa , aac(6′)/aph(2′) and ant(4′)-Ia genes among K. pnuemoniae. However, a study carried out in India by Chaudhary and payasi(2014) they mention that aminoglycoside modifying enzyme including aac(6), ant(2) and aph(3) among gram negative pathogens were 75.7%.

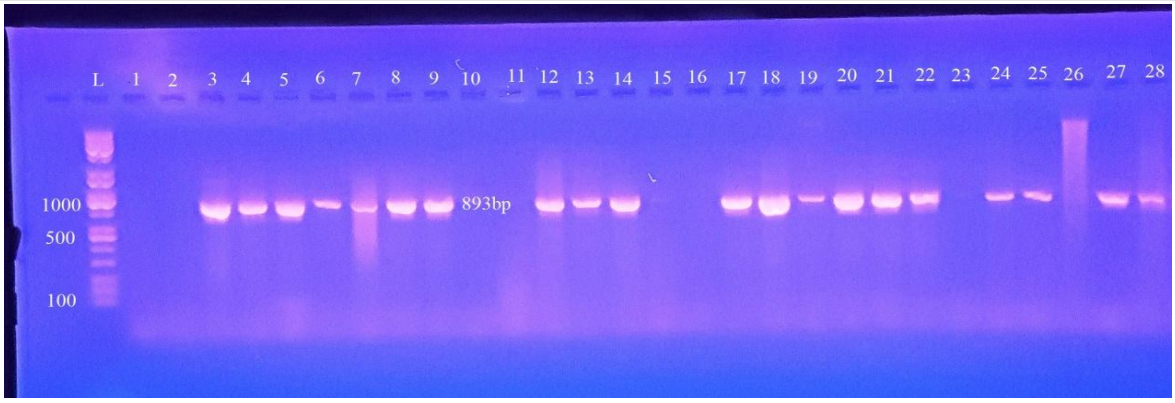


Figure (1): Gel electrophoresis of PCR products for DNA of 28 K.pneumoniae isolated from hospital environment using strA-strB primer appear positive band at 893 bp. Line L, molecular size marker, products migrated at 80 volt for 80 minutes and stained by ethidium bromide

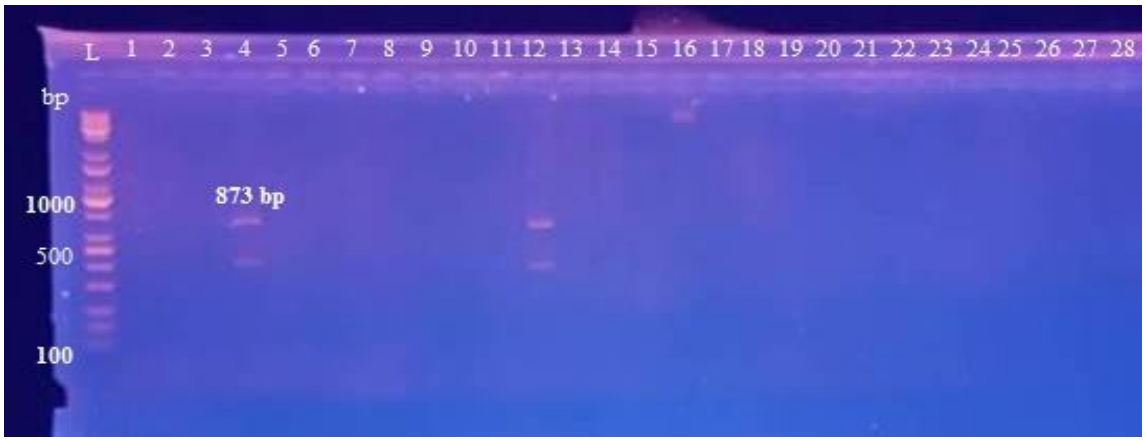


Figure (2): Gel electrophoresis of PCR products for DNA of 28 K.pneumoniae isolated from hospital environment using aacC-1 primer apear positive band at 873 bp. Line L, molecular size marker, products migrated at 80 volt for 80 minutes and stained by ethidium bromide

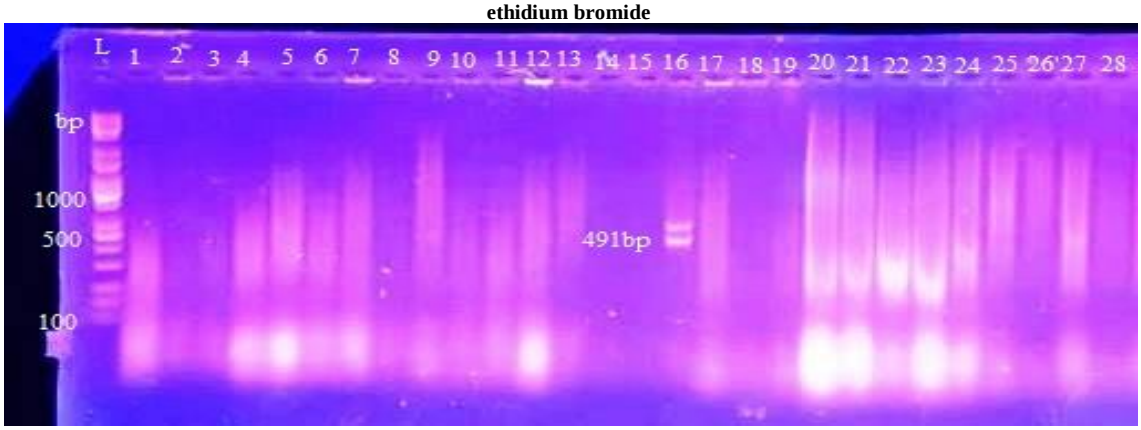


Figure (3): Gel electrophoresis of PCR products for DNA of 28 K.pneumoniae isolated from hospital environment using aac(6′)/aph(2′) primer apear positive band at 491 bp. Line L, molecular size marker, products migrated at 80 volt for 80 minutes and stained by ethidium bromide.

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